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Investigation of effects of metformin on rats' urinary bladder contractions

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Abstract

Aim: The effects of different doses of metformin on the contractility of female Sprague-Dawley rat bladder strips were evaluated.

Materials and Methods: Rats were decapitated and the bladder strips measuring 2–4 × 6–12 mm prepared from the whole urinary bladder were suspended in isolated tissue organ baths. Tissues in the chambers were rinsed every 30 minutes for 60 minutes and allowed to equilibrate. Afterwards, 10^{-4}M acetylcholine was added to all isolated tissue baths. After one hour of rinsing, metformin (2 mM) was added and incubated 30 minutes. After 30 minutes, the same dose of acetylcholine was added without rinsing, and immediately after this protocol was performed with 10 mM and 20 mM metformin doses.

Results: All of these doses led to increased contractions in a dose-dependent manner. The 2 mM dose did not induce any contraction in area (213.67±33.10 %) compared to control, but there was a significant increase in peak-to-peak amplitude (127.65±4.24 %, p=0.009). In addition, the peak-to-peak amplitude was significantly increased at the 10 mM and 20 mM doses (163.86±8.57 % and 162.58±6.76 %, p=0.0001, respectively) compared to the control and there was a statistically significant difference. In addition, the area value increased significantly compared to control at the 20 mM dose alone (3767.64±733.70 %, p=0.0001).

Conclusion: Our present data show that the treatment of varied high doses of metformin may lead to the increase of contractions at ex vivo isolated tissue bath. Therefore, this effect can escalate some symptoms of diabetic bladder dysfunction.

Introduction

Metformin is a biguanide derived from Galega officinalis and has been used in the treatment of diabetes for more than 60 years. The first choice in the treatment of type-2 diabetes (T2DM) has been found to be a low cost, safety profile and its effect of reducing hepatic gluconeogenesis and improving insulin sensitivity [1]. Although metformin does not reduce insulin secretion, it is a drug that lowers serum glucose levels through a variety of non-pancreatic mechanisms. It is called "insulin sensitizer" because it increases the effects of insulin. Metformin has no significant side effects, but can cause many symptoms including muscle pain, fatigue, lactic acidosis, dizziness, fast/difficult breathing, tremors, slow/irregular heartbeat, blue/cold skin, stomach pain with diarrhea and nausea [2]. The most common side effects are gastrointestinal symptoms and the incidence rate is between 20-30%. The most serious side effect is lactic acidosis and the incidence rate is 1/30.000 [3, 4]. Diabetes mellitus is a major global health threat in terms of its incidence and complications. T2DM and its complications have contributed greatly to the burden of mortality and disability worldwide. More than 90% of cases of diabetes mellitus are T2DM [5]. Diabetic bladder dysfunction (DBD) is a number of bladder-related symptoms in diabetic patients. T2DM is a disease with various symptoms, including diabetic bladder dysfunction and cystitis. The prevalence of DBD in diabetic patients is 43-87%. Clinical symptoms include overactive bladder and urge incontinence, decreased sensation, increased capacity and voiding problems. Diabetes is a condition that affects up to one in five people in the world. DBD is not a life-threatening condition, but it has very damaging effects on quality of life for sufferers and their families [6]. Neuronal

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control of the function of the bladder includes advanced and complex interaction between the autonomic and somatic afferent and efferent pathways. A study has shown that diabetes-induced peripheral neuropathy and urinary bladder dysfunction can cause kidney failure in people with diabetes [7]. Activation of muscarinic, cholinergic and postganglionic pelvic nerve fibers leads to the contraction of the bladder. The bladder is one of the largest organ in the human body and can be affected by urinary tract infections. The symptoms include weak stream, dribbling and hesitancy in micturition. Symptoms are caused by damage to the pelvic nerve fibers reaching to the urinary bladder [8]. Although metformin is the most commonly used antidiabetic for T2DM, there are not enough studies investigating the efficacy of metformin on bladder dysfunction, a major complication of diabetes. Considering this gap, in this study, we aimed to investigate the efficacy of metformin on bladder contractile activity which has an important role in bladder dysfunction. With this study, we think that this gap in the literature will be eliminated.

Materials and Methods

Animals

A group of rats weighing 250-300 grams have been used in a new research project at the University of ----- 12 adult (3-5 months old) female Sprague-Dawley rats were obtained from the University (-----, -----). The number of animals included in the study was planned as 12 because at least 7 rats are required for statistical analysis, possible situations such as illness or death of rats occur, and we want to increase the number of samples. Female rats included in the study were randomly selected from among female rats that we are sure to have regular cycles. Experiments were approved by the Ethics Committee of Firat University. While the rats were decapitated to have their uterus removed, no anesthesia was administered before decapitation, considering the possibility that it might affect the contraction mechanism. During the use of experimental animals, ethical guidelines for the use of laboratory animals were taken into account and there was no violation of ethical rules.

Research ethics

The Firat University, Animal Experiments Local Ethics Committee, permission number 124, with protocol number 2018/18 dated 27/06/2018, evaluated and approved this study procedure.

Drugs

Acetylcholine chloride (Sigma-Aldrich, -----) and metformin were the medicines used (Bilim Pharmaceuticals, -----). Metformin pills containing 1000 mg metformin hydrochloride were obtained from a nearby pharmacy to mimic human consumption. Metformin hydrochloride was crushed into powder and dissolved in Krebs-Henseleit solution. Metformin solution was prepared and used just before starting the experiment.

Preparations

Rats were decapitated by guillotine to investigate the functional role of metformin in the bladder. The abdominal wall of the decapitated rats was cut at the midline and the abdomen was opened. The urinary bladder was then taken in its entirety, cleaned of fat and other tissues, and placed in Krebs-Henseleit solution (composition in mM: NaCI 118, KH_2PO_4 1.2, $MgSO_4$ 1.2, EDTA 0.03, $CaCl_2$ 1.25, KCl 4.7, $NaHCO_3$25, glucose 11) before being shaped into a flat sheet. After that, sheets measuring 2–4 x 6–12 mm were cut into longitudinal strips. A needle was used to secure each strip to a petri plate containing solid paraffin. Tissue strips were then sutured and placed in isolated tissue baths (5 mL, 10 mL, and 20 mL) containing Krebs-Henseleit solution. After the tissue strips were placed in the baths, they were bubbled with 95% 02/5% CO2 at 37°C. The tension created by the tissues was measured using the MP150 instrument's transducers (Biopac Systems, Inc., U.S.A.).

Protocol

Before starting the experiment, 1.5 g of resting tension was administered to all tissues. Tissues in the isolated tissue bath were rinsed every 30 minutes and allowed to equilibrate for 60 minutes. Following equilibration, different concentrations of acetylcholine (10^{-9}, 10^{-8}, 10^{-7}, 10^{-6}, 10^{-5}, 10^{-4}M) were tested, with the 10^{-4}M concentration showing the best reaction and being added to all baths. 2 mM metformin was given after one hour of rinsing and incubated for 30 minutes. After 30 minutes, the same dose of acetylcholine was administered without rinsing, and the procedure was repeated with 10 mM and 20 mM metformin doses.

Statistical analysis

SPSS version 22 was used for all statistical analysis (IBM Corp, Inc., Authorization code: 794f5c72bc41572d732f , Chicago, IL, USA). The number of animals to be used in the experiments; 8% deviation, type 1 error (α) 0.05 and type 2 error (β) (Power=0.80) and at least 12 animals were determined by power analysis. The mean and standard error of the mean (±SE) were used to represent the data (n=12). For peak to peak amplitude and area values, the control group values were recognized as 100 percent, and the change was computed as percent (percent) for all treatment groups. The data were statistically analyzed using the one-way ANOVA test. All results with p value less than 0.05 in all analyzes were considered statistically significant.

Results

The 2 mM dose had no effect on area (213.67 ± 33.10% p=0.997) values (Fig. 1-B), but it did have a significant increase in peak to peak amplitude (127.65 ± 4.24%, p=0.009) values (Fig. 1-A). In addition, when compared to control, peak to peak amplitude in 10 mM and 20 mM (163.86 ± 8.57% and 162.58 ± 6.76%, respectively, p=0.0001) was substantially higher (Fig. 1-A). Furthermore, when compared to control, the area grew significantly at only 20 mM (3767.64 ± 733.70%, p=0.0001) (Fig. 1-B).

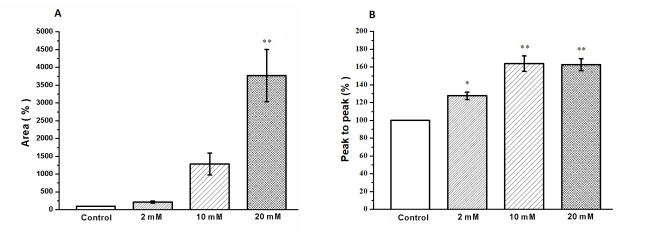

Figure 1. (A) Effects of metformin on contractions of the urinary bladder strips. The area of strips was increased by metformin at only 20 mM dose (*p < 0.001; n = 12). (B) Effects of metformin on contractions of the urinary bladder strips. The peak to peak amplitude of strips was increased by metformin at 2, 10 and 20 mM doses (*p<0.01, **p<0.001; n = 12).

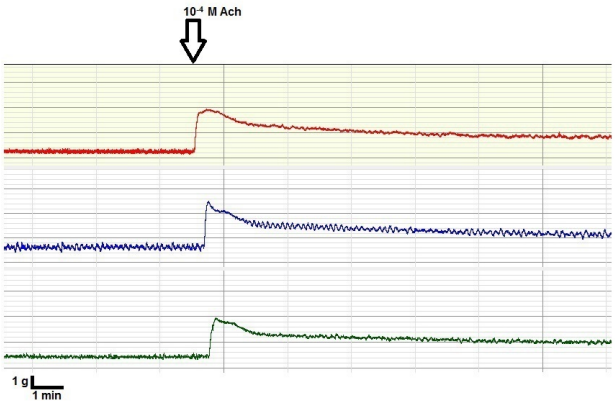

Figure 2. Contractile response pattern to acetylcholine (Ach) (10^{-4}M) in the isolated urinary bladder strips. This example show recording of muscle contraction from three recording for the control.

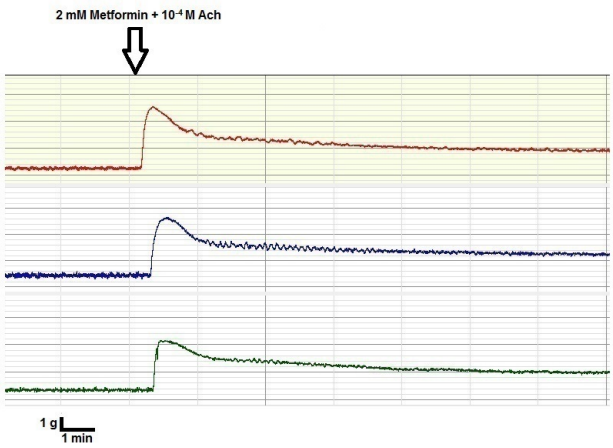

Figure 3. Contractile response pattern to acetylcholine (Ach) (10^{-4}M) and metformin (2 mM) in the isolated urinary bladder strips. This example show recording of muscle contraction from three recording for the 2 mM metformin treatment.

Figure 4. Contractile response pattern to acetylcholine (Ach) (10^{-4}M) and metformin (10 mM) in the isolated urinary bladder strips.This example show recording of muscle contraction from three recording for the 10 mM metformin treatment.

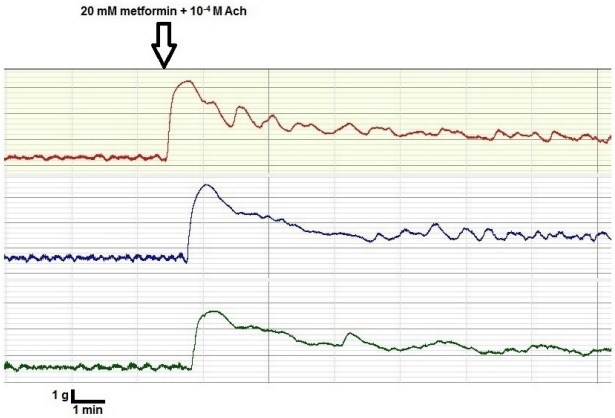

Figure 5. Contractile response pattern to acetylcholine (Ach) (10^{-4}M) and metformin (20 mM) in the isolated urinary bladder strips. This example show recording of muscle contraction from three recording for the 20 mM metformin treatment.

Discussion

Effects of different metformin doses (2, 10 and 20 mM) on the rat urinary bladder strips contractility was established in this study. Our data showed that metformin increases bladder contractions at ex vivo (Fig. 3, 4, 5). According to the United States Centers for Disease Control and Prevention (CDC), disease affects 25.8 million people in the United States, with T2DM accounting for 90–95 percent of cases [7]. Diabetics have a lot of urologic complications, including DBD. DBD or diabetic cystopathy is the most common one among this complications. DBD has a wide range of symptoms, like bladder overactivity, impaired bladder contractility, and areflexic bladder. Even though DBD is not fetal for life, it leads to a dramatic decrease in life quality [7]. Metformin has been used in the treatment of diabetes for a long time [1]. With regard

to metformin effect on the bladder contractility, there were only few meeting reports and there was insufficient studies on the mechanism of action of metformin on the urinary bladder of rats. [9, 10, 11]. Studied that differential effects of metformin in rats' urinary bladder and other different tissues. As compatible with our study results, metformin led to increased acetylcholine-induced contractions (Fig. 2, 3, 4, 5). In spite of this, in another study of the same researcher, metformin (100 µM) decreased acetylcholine-induced contractions of isolated rat urinary bladder strips [12]. This decrement may result from the (4-hour) pretreatment with metformin and the application of a much lower dose compared to ours. Despite this, in the other study of the same researcher [13], they suggested that levels $\leq$ 200 micromolar had not interfered with the ability of bethanechol to contract urinary bladder contractions. The discrepancy between the two studies might result from contraction stimulating agents types and it being a human study. In another study in the literature, metformin was found to improve nerve conduction of acetylcholine. In the same study, it was seen that it increased acetylcholine sensitivity in muscle tissue and provided its restoration [14]. In another study, it was determined that an increase in acetylcholine in smooth muscle induces contraction by increasing Ca^{+2}release [15].

In this study, we used a dose, which was close to human therapeutic dose. By this way, we were able to determine closest effects of metformin to a human on the rat urinary bladder contractions. Studied the effects of metformin on the contractions of isolated rat urinary bladder [16]. They employed a double knockout [DKO] animal model with hepatic-specific insulin receptor substrate 1 and 2 deletions to ameliorate T2DM. The detrusors in these animals' bladders were overactive. It was known that tumor necrosis factor (TNF) superfamily genes expression was increased in DKO mice bladders [16]. They discovered that TNF- was upregulated in DKO mice's serum and bladder smooth muscle tissue. TNF- caused primary cultured bladder smooth muscle cells to contract more. TNF-upregulated Rho kinase activity and phosphorylated myosin light chain, according to this study, which boosted smooth muscle cell contractions. When TNF-α was applied to these cells with metformin, it was shown that they improved diabetic bladder dysfunction with together. In a study on the effects of cyclophosphamide and metformin on bladder dysfunction, it was revealed that metformin partially inhibited cycloffamide-induced cystitis [10]. When cyclophosphamide and metformin were combined, increased cytotoxicity was observed. Application of meformin in the right dose and with the right application method can positively change its effects on the bladder. However, in our study, metformin increased rats' urinary bladder contractions. This difference may be resulted due to experimental design and it being an in vitro study. Other difference between the two studies was that we did not apply TNF-α and our results indicated that only metformin effected at ex vivo. Metformin might have shown synergistic effect with TNF-α and the opposite was also possible when it was applied on its own. At another study, it was suggested that metformin led to a decrease in obese mice bladder hypercontractility [17]. In this study, they used to carbachol and other different agents to stimulate contractions. The discrepancy between our study and this study might originate because of a different animal model and the mechanism of action of carbachol. It was suggested that carbachol is the most powerful parasympathetic agent with a longer duration of action [18] and this situation was seen before at another urinary bladder study [19]. In addition to this, they used just 1 µM metformin dose while we used 2, 10 and 20 mM doses. As it is seen before [12], low dose metformin could decrease bladder contractility in rodents.

Conclusion

Different from other studies, we used doses close to the metformin treatment doses on human. This allowed us to find the dose of metformin that had the most influence on the contraction activity of the human bladder. According to our results, while using metformin in patients with bladder dysfunction which is observed as a complication of diabetes, enhancing the effect of metformin on bladder contractile activity should be considered. Further studies must be conducted to elucidate the underlying pathophysiological mechanisms of the enhancing effect of metformin on bladder contractile activity.

Declaration of interest

The authors contributing to this research declare that there is no conflict of interest that could harm the objectivity of the research.

Data availability

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Author contribution statement

EK and IS designed and supervised the study. AY, NU, and SS conducted all animal experiments. GZ, FT and BH contributed to data analysis. EK wrote the manuscript, and all authors approved the final manuscript.

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Ethics approval

This study protocol was reviewed and approved by Firat University, Animal Experiments local Ethics Committee, approval number 124, with protocol number 2018/18 dated 27/06/2018.

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